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Taxonomic Studies of *Deutzia* (Saxifragaceae, s. l.) in Japan 2. Pollen Grains

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The morphology, exine structure, size and fertility of the pollen grains of 8 species, 5 varieties and 1 form of the genus *Deutzia* from Japan were examined with light and scanning electron microscopy. The results indicated that the pollen grains are 3-colporate, prolate spheroidal or subprolate, semitectate, reticulate or microreticulate with tectum, and the shape, tectum-pattern and structure of pore of the pollen grains are similar in all examined taxa. The pollen grains have a high stainability over 90 %. A correlation between the pollen size and the ploidy level was found. The value of pollen characters for taxonomic purposes and position of taxa studied on *Deutzia* were discussed.

Key words: Correlation between pollen size and ploidy level, *Deutzia*, pollen morphology, taxonomy

Introduction

The pollen morphology of the genus *Deutzia* has been studied by a number of botanists, but the comprehensive and systematic study of a large number of species still remain to be investigated. On the other hand, it is known in some flowering plants that the cytological status may be reflected in morphological characters of pollen grains, viz., size, shape, polarity, arrangement and number of apertures (Erdtman 1969). There have been some papers on pollen size variation attributed to polyploid, most of which show increase of pollen size is correlation with polyploidy. In *Rodgersia* (Saxifragaceae), Akiyama et al. (1990) used pollen size to detect ploidy level in the herbarium specimens. Funamoto and Nakamura (1994) reported the chromosome number, size of pollen grains, stainability, and cytogeographical distribution of *Deutzia crenata*, and showed that the size of pollen grains was a significant difference between the two cytotypes. However, the great majority of the Japanese species, the correlation between pollen size and ploidy level has not been examined.

This paper provides a number of characters of pollen grains for discussing whether the pollen morphology offers a support to species delimitation and show the correlation with ploidy level.

Materials and Methods

The pollen grains were obtained from our collections and specimens deposited in the Herbarium, University of Tokyo (TI) (Table 1). The vouchers were deposited in TI.

All the samples were acetolysed according to Erdtman (1960). For observations using a scanning electron microscope (SEM), acetolysed pollen grains were dehydrated, critical point dried, coated by goldpalladium, and examined with HITACHI S-4500 scanning electron microscope, accelerator Voltage at 15kv. The exine structure was studied on fractures of acetolysed pollen grains. The fractures were produced by KAIJODENKI TA-4280 ultrasonic generator.

For observations using a light microscope (LM), thirty acetolysed pollen grains were measured for the polar and equatorial axes under the Nikon AFM-B light microscope (under immersion oil at a × 1000 magnification). The ratio between polar and equatorial and the mean P/E ratio were calculated from their individual values. The stainability of the pollen grains was examined with a 1 % solution of cotton blue-lactophenol. Five hundreds pollen grains were measured.

The palynological terminology follows Punt et al. (1994); the shape was determined by the equatorial view and classified by the criteria adopted from Erdtman (1952). The taxonomic treatment essentially follows Ohba (1989).

Results and Discussion

Observations on the pollen morphology:

Pollen size is expressed by polar length × equatorial length in the following descriptions. The pollen grains are all 3-colporate, prolate spheroidal or subprolate, semitectate, and reticulate or microreticulate (Table 2). In all examined taxa, the pollen grains are stained well (over 90 %).

Table 1. Origin of the *Deutzia* materials observed

Taxon	Locality	Collection no.
D. bungoensis	Oita Prefecture, Hasama-cho, along the bank of Yufu River	Niu 98632020
D. crenata var. *crenata* f. *crenata*	Ehime Prefecture, Ipponmatus-cho, Sasayama	Niu 975138
D. crenata var. *crenata* f. *pubescens*	Nagano Prefecture, Shimoinagun, Yasuokamura, Kinno	Kazuo & Asano s. n.
D. crenata var. *floribunda*	Oita Prefecture, Takeda City, Kobaru Keikoku	Niu 986222
D. gracilis var. *gracilis*	Tochigi Prefecture, Nikko City, Nikko Botanical Garden	Shinozaki & Peki s. n.
D. gracilis var. *microcarpa*	Miyazaki Prefecture, Hinokage-cho, Tokawadake	Minamitanii & Niu 994304
D. gracilis var. *ogatae*	Ehime Prefecture, Tsushima-cho Nametoko Keikoku	Niu 98646
D. gracilis var. *zentaroana*	Kumamoto Prefecture, Itsuki-mura, Ikenozuru	Otomasu & Niu 984224
D. maximowicziana	Ehime Prefecture, Kuma-cho, Omogoukei	Niu 975141
D. naseana var. *naseana*	Amami Oshima Island, Setouchi-cho	Ohba & Akiyama 9803055
D. scabra var. *scabra*	Tokyo Prefecture, Okutama-cho, Unazawa Keikoku	Niu 9852016
D. scabra var. *sieboldiana*	Kumamoto Prefecture, Itsuki-mura, Kyuorise	Otomasu & Niu 9842225
D. uniflora	Saitama Prefecture, Chichibu Mitsumineguchi	Hara s. n.
D. yaeyamensis	Ryukyu, Iriomote Island	Anki s. n.

1. *Deutzia crenata* Siebold & Zucc. var. *floribunda* (Nakai) H.Ohba (Figs. 1–4)

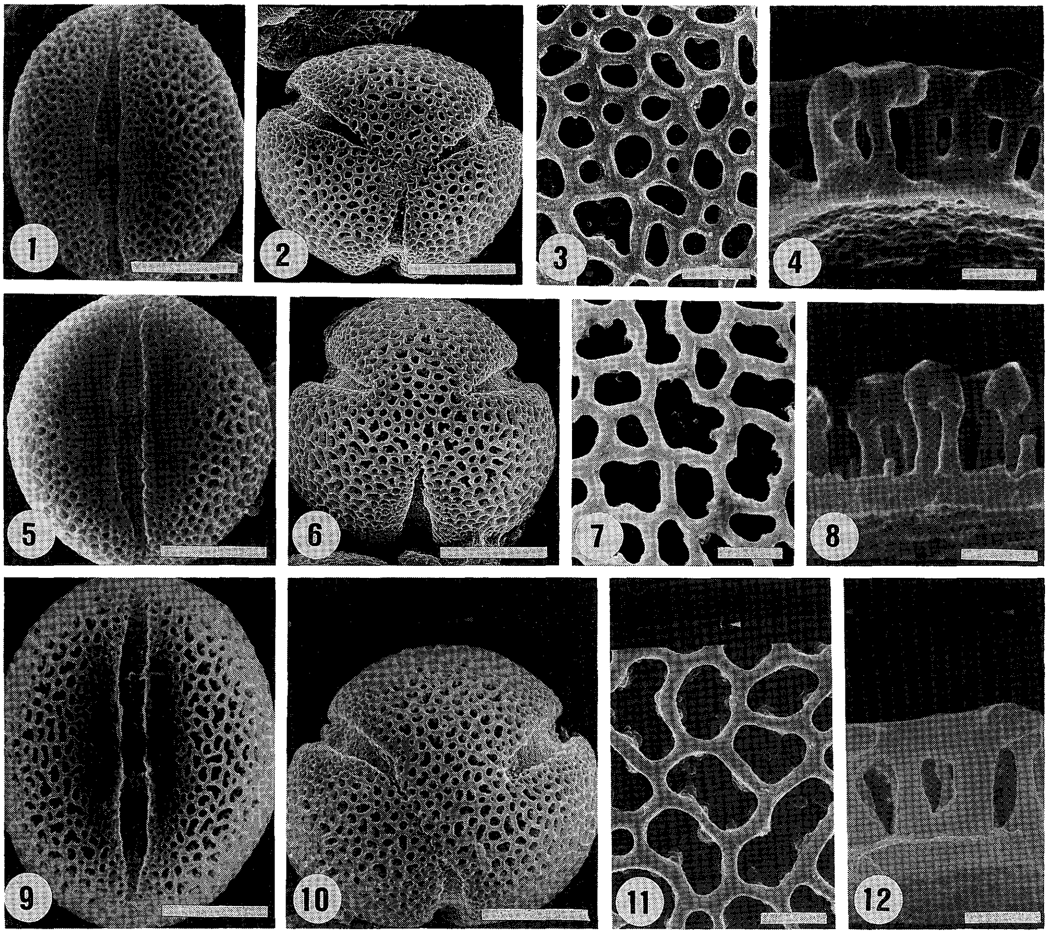
Spheroidal (P/E ratio: 1.08), polar axis 17.0–(18.3)–20.1 μm, equatorial axis 14.5–(16.9)–18.2 μm. Exine 1.0–1.5 μm thick; columellae unbranched or indistinctly branched; semitectate. Tectum-pattern reticulate or microreticulate (lumina size mostly > 1 μm).

2. *Deutzia crenata* Siebold & Zucc. var. *crenata* f. *pubescens* (Makino) H.Hara (Figs. 5–8)

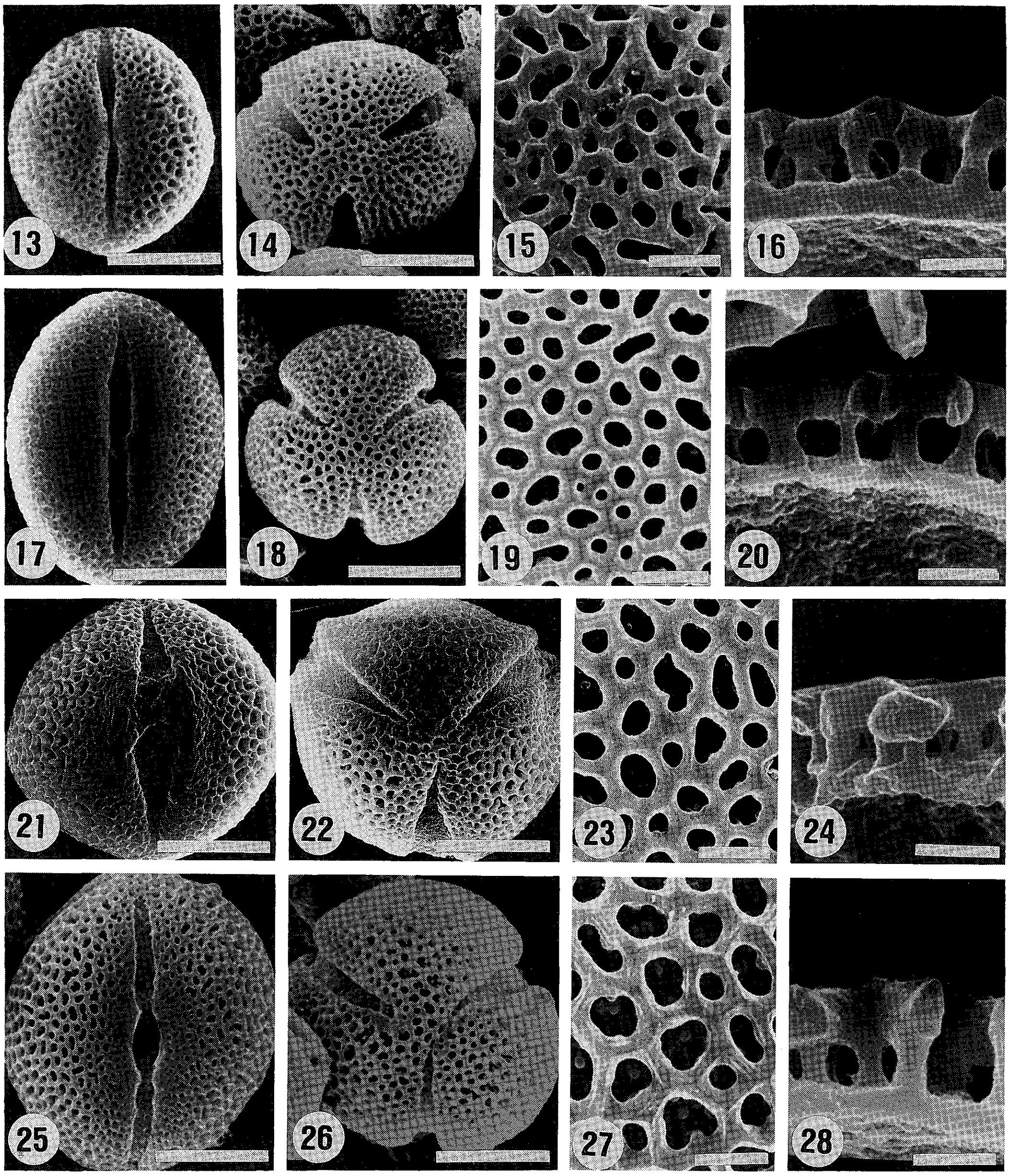
Spheroidal (P/E ratio: 1.03), polar axis 16.9–(19.0)–21.0 μm, equatorial axis 16.8–(18.4)–19.4 μm. Exine 0.8–0.9 μm thick; columellae unbranched, semitectate. Tectum-pattern reticulate (lumina size < 1μm).

3. *Deutzia crenata* Siebold & Zucc. var. *crenata* f. *crenata* (Figs. 9–12)

Spheroidal (P/E ratio: 1.05), polar axis



Figs. 1–12. SEM micrographs of pollen grains, pollen grain surfaces and section (fracture) of exine. 1–4. *Deutzia crenata* var. *floribunda* (1. equatorial view, 2. polar view, 3. detail of ornamentation, 4. section of exine). 5–8. *D. crenata* var. *crenata* f. *pubescens* (5. equatorial view, 6. polar view, 7. detail of ornamentation, 8. section of exine). 9–12. *D. crenata* var. *crenata* f. *crenata* (9. equatorial view, 10. polar view, 11. detail of ornamentation, 12. section of exine). Scale bar (Figs. 1, 2, 5, 6, 9, 10) = 5 μm, Scale bar (Figs. 3, 7, 11) = 1 μm, Scale bar (Figs. 4, 8, 12) = 0.5 μm.



Figs. 13–28. SEM micrographs of pollen grains, pollen grain surfaces and section (fracture) of exine. 13–16. *Deutzia gracilis* var. *microcarpa* (13. equatorial view, 14. polar view, 15. detail of ornamentation, 16. section of exine). 17–20. *D. gracilis* var. *gracilis* (17. equatorial view, 18. polar view, 19. detail of ornamentation, 20. section of exine). 21–24. *D. gracilis* var. *zentaroana* (21. equatorial view, 22. polar view, 23. detail of ornamentation, 24. section of exine). 25–28. *D. gracilis* var. *ogatae* (25. equatorial view, 26. polar view, 27. detail of ornamentation, 28. section of exine). Scale bar (Figs. 13, 14, 17, 18, 21, 22, 25, 26) = 5 µm, Scale bar (Figs. 15, 19, 23, 27) = 1 µm, Scale bar (Figs. 16, 20, 24, 28) = 0.5 µm.

20.5–(22.2)–24.2 μm, equatorial axis 17.9–(21.1)–23.1 μm. Exine 1.5–2.5 μm thick; columellae unbranched or indistinctly branched, semitectate. Tectum-pattern reticulate (lumina size > 1μm).

4. *Deutzia gracilis* Siebold & Zucc. var. *microcarpa* Hatus. (Figs. 13–16)

Spheroidal (P/E ratio: 1.05), polar axis 15.5–(16.7)–17.9 μm, equatorial axis 14.9–(15.9)–17.1 μm. Exine 0.7–0.8 μm thick; columellae unbranched, semitectate. Tectum-pattern microreticulate (lumina size < 1μm).

This variety was provisionally proposed by Hatusima (1989), which was found only in Oita and Miyazaki prefectures in Kyushu.

5. *Deutzia gracilis* Siebold & Zucc. var. *gracilis* (Figs. 17–20)

Spheroidal (P/E ratio: 1.05), polar axis 16.0–(17.5)–19.2 μm, equatorial axis 15.5–(16.7)–18.5 μm. Exine 0.7–0.8 μm thick; columellae unbranched, semitectate. Tectum-pattern microreticulate (lumina size < 1 μm).

6. *Deutzia gracilis* Siebold & Zucc.var. *zentaroana* (Nakai) Hatus. (Figs. 21–24)

Spheroidal (P/E ratio: 1.02), polar axis 17.3–(18.2)–19.7 μm, equatorial axis 17.1–(17.9)–19.5 μm. Exine 0.6–0.7 μm thick; columellae unbranched, semitectate. Tectum-pattern microreticulate (lumina size < 1 μm).

7. *Deutzia gracilis* Siebold & Zucc. var. *ogatae* (Koidz.) Ohwi (Figs. 25–28)

Spheroidal (P/E ratio: 1.06), polar axis 17.5–(19.4)–21.5 μm, equatorial axis 16.2–(18.3)–20.2 μm. Exine 0.7–0.8 μm thick; columellae unbranched; semitectate. Tectum-pattern reticulate or microreticulate (lumina size mostly > 1μm).

8. *Deutzia scabra* Thunb. var. *scabra* (Figs. 29–32)

Spheroidal (P/E ratio: 1.02), polar axis 14.8–(15.5)–16.8 μm, equatorial axis 14.5–(15.2)–16.1 μm. Exine 0.6–0.7 μm thick; columellae unbranched; semitectate. Tectum-pattern, microreticulate (lumina size < 1 μm).

9. *Deutzia scabra* Thunb. var. *sieboldiana* (Maxim.) H.Hara (Figs. 33–36)

Spheroidal (P/E ratio: 1.02), polar axis 14.8–(15.6)–17.0 μm, equatorial axis 14.5–(15.2)–16.0 μm. Exine 0.7–0.8 μm thick; columellae unbranched or indistinctly branched; semitectate. Tectum-pattern, microreticulate (lumina size < 1 μm).

10. *Deutzia. bungoensis* Hatus. (Figs. 37–40)

Spheroidal (P/E ratio: 1.04), polar axis 15.2–(17.1)–18.5 μm, equatorial axis 14.8–(16.5)–18.0 μm. Exine 0.6–0.8 μm thick; columellae unbranched; semitectate. Tectum-pattern, reticulate (lumina size > 1 μm).

11. *Deutzia maximowicziana* Makino (Figs. 41–44)

Spheroidal (P/E ratio: 1.02), polar axis 15.4–(16.6)–18.0 μm, equatorial axis 15.2–(16.4)–17.9 μm. Exine 1.0–2.0 μm thick; columellae unbranched or indistinctly branched; semitectate. Tectum-pattern, reticulate (lumina size > 1 μm).

12. *Deutzia naseana* Nakai var. *naseana* (Figs. 45–48)

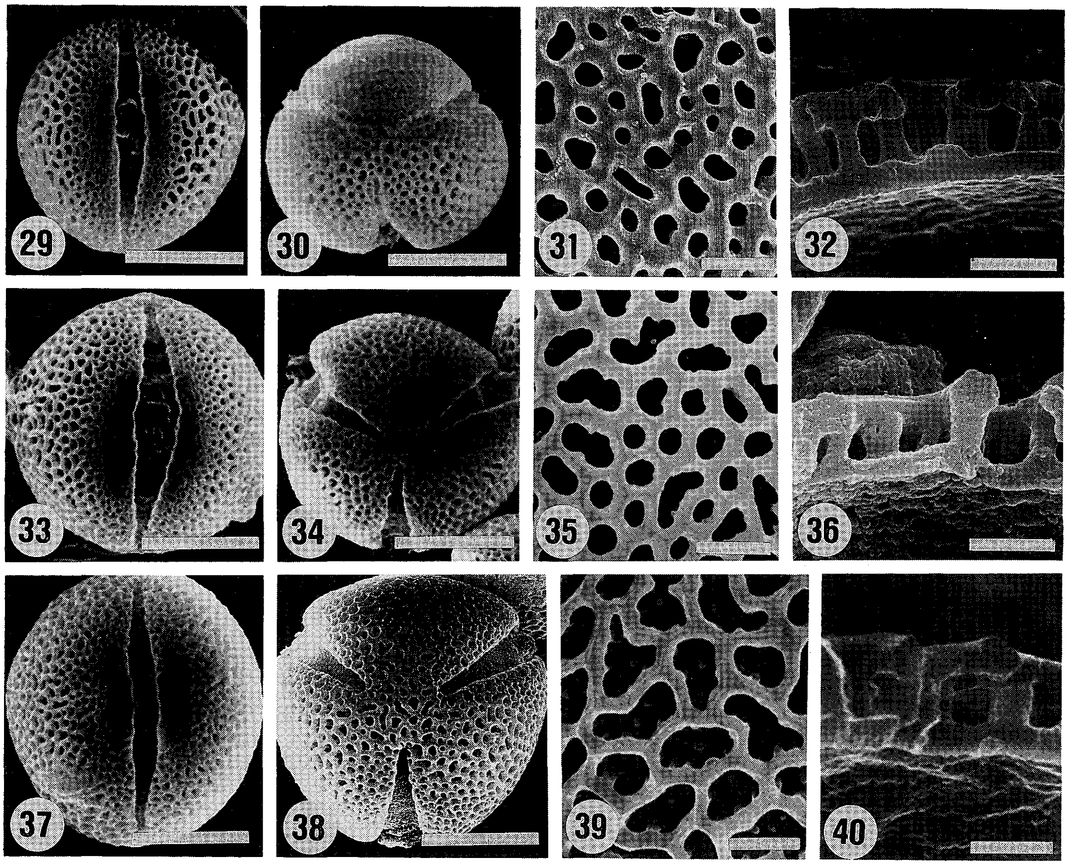
Spheroidal (P/E ratio: 1.05), polar axis 16.0–(17.5)–19.0 μm, equatorial axis 15.2–(17.2)–18.8 μm. Exine 1.5–2.0 μm thick; columellae unbranched or indistinctly branched; semitectate. Tectum-pattern, reticulate (lumina size > 1 μm).

13. *Deutzia yaeyamensis* Ohwi (Figs. 49–52)

Spheroidal (P/E ratio: 1.13), polar axis 16.0–(17.3)–18.1 μm, equatorial axis 14.2–(15.4)–17.1 μm. Exine 0.8–0.9 μm thick; columellae unbranched; semitectate. Tectum-pattern, reticulate or microreticulate (lumina size mostly > 1 μm).

14. *Deutzia uniflora* Shirai (Figs. 53–56)

Subprolate (P/E ratio: 1.23), polar axis 19.9–(21.5)–23.1 μm, equatorial axis 16.1–(17.5)–18.9 μm. Exine 0.6–0.7 μm thick; columellae unbranched or indistinctly branched; semitectate. Tectum-pattern, reticulate

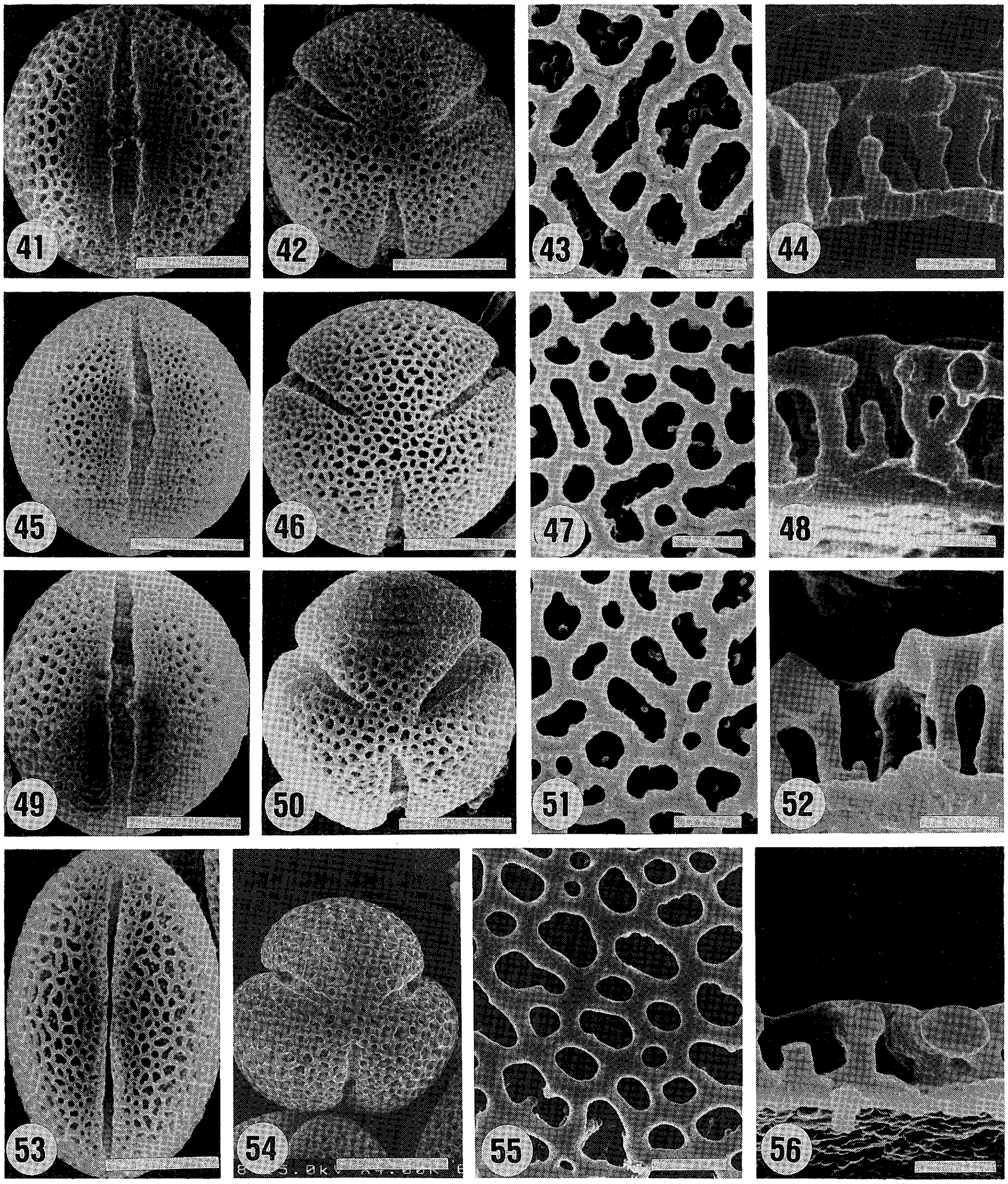


Figs. 29–40. SEM micrographs of pollen grains, pollen grain surfaces and section (fracture) of exine. 29–32. *Deutzia scabra* var. *scabra* (29. equatorial view, 30. polar view, 31. detail of ornamentation, 32. section of exine). 33–36. *D. scabra* var. *sieboldiana* (33. equatorial view, 34. polar view, 35. detail of ornamentation, 36. section of exine). 37–40. *D. bungoensis* (37. equatorial view, 38. polar view, 39. detail of ornamentation, 40. section of exine). Scale bar (Figs. 29, 30, 33, 34, 37, 38) = 5 μm, Scale bar (Figs. 31, 35, 39) = 1 μm, Scale bar (Figs. 32, 36, 40) = 0.5 μm.

or microreticulate (lumina size mostly > 1 μm).

Correlation between pollen size and ploidy level:

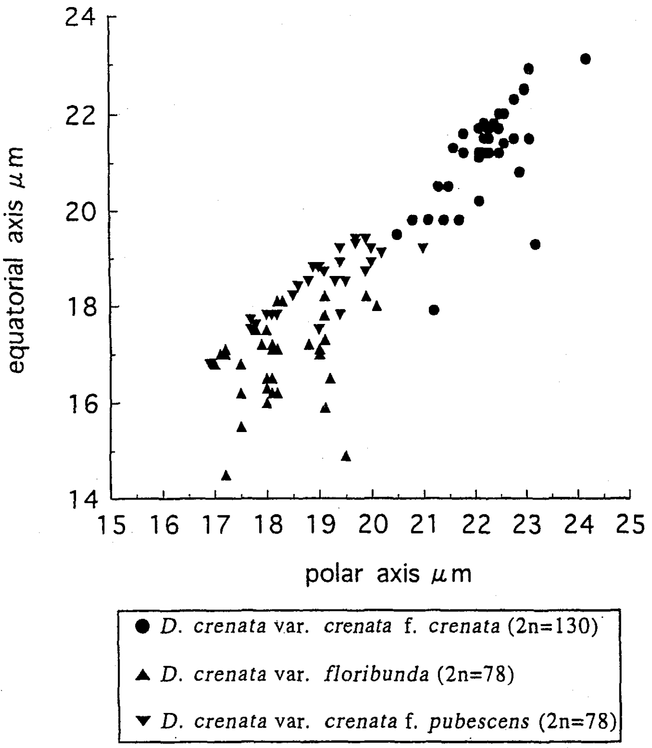
The inter- and intraspecific taxa with higher ploidy level have a tendency to have larger pollen grains (Figs. 57, 58). Particularly in the intraspecific taxa of *D. crenata* and *D. gracilis* the tendency is conspicuous. In six diploid taxa, *D. gracilis* var. *gracilis*, *D. gracilis* var. *microcarpa*, *D. maximowicziana*, *D. scabra* var. *scabra*, *D. scabra* var. *sieboldiana* and *D. yaeyamensis*, the mean of length of polar axis ranges from 15.5 μm to 17.5 μm and that of equatorial axis ranges from 15.2 μm to 16.7 μm; in three tetraploid, *D. bungoensis*, *D. gracilis* var. *zentaroana* and *D. naseana* var. *naseana*, polar axis; 17.1 μm to 18.2 μm and equatorial axis; 16.5 μm to 17.9 μm; in two hexaploid taxa, *D. crenata* var. *crenata* f. *pubescens* and *D. crenata* var. *floribunda*, polar axis; 18.3 μm to 19.0 μm

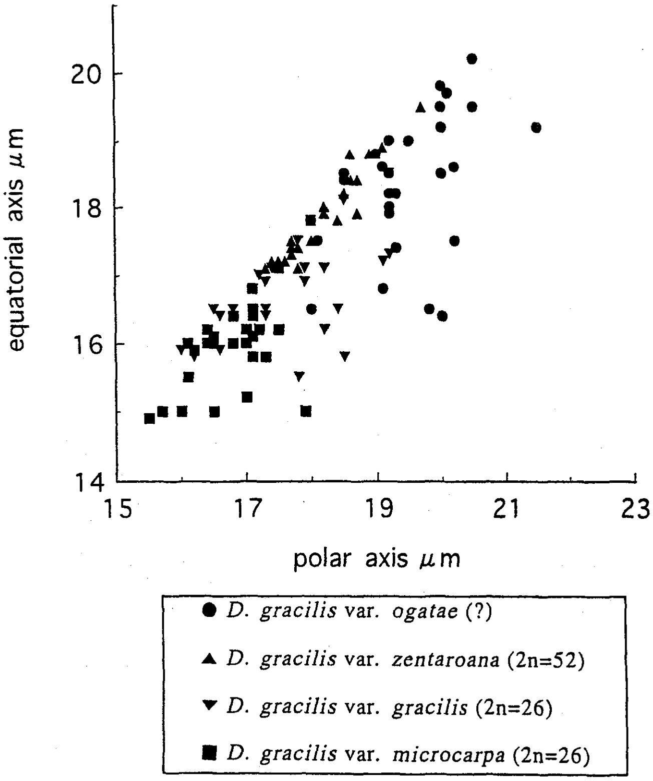


Figs. 41–56. SEM micrographs of pollen grains, pollen grain surfaces and section (fracture) of exine. 41–44. *Deutzia maximowicziana* (41. equatorial view, 42. polar view, 43. detail of ornamentation, 44. section of exine). 45–48. *D. naseana* var. *naseana* (45. equatorial view, 46. polar view, 47. detail of ornamentation, 48. section of exine). 49–52. *D. yaeyamensis* (49. equatorial view, 50. polar view, 51. detail of ornamentation, 52. section of exine). 53–56. *D. uniflora* (53. equatorial view, 54. polar view; 55. detail of ornamentation, 56. section of exine). Scale bar (Figs. 41, 42, 45, 46, 49, 50, 53, 54) = 5 µm, Scale bar (Figs. 43, 47, 51, 55) = 1 µm, Scale bar (Figs. 44, 48, 52, 56) = 0.5 µm.

Table 2. The characteristics of pollen grains size of polar axis and equatorial axis and stainabilities of Japanese taxa of *Deutzia*. Chromosome numbers (2n) are shown in parentheses after taxon name

Taxon (2n)	Aperture	Shape	Tectum-pattern	Exine	Polar axis (µm)				Equatorial axis (µm)				P/E	Stainability (%)
					max	min	Mean	SD	max	min	Mean	SD		
D. bungoensis (52)	3-colporate	prolate spheroidal	reticulate	semitectate	18.5	15.2	17.1	0.81	18.0	14.8	16.5	0.77	1.04	97.0
D. crenata var. *crenata* f. *crenata* (130)	3-colporate	prolate spheroidal	reticulate	semitectate	24.2	20.5	22.2	0.75	23.1	17.9	21.1	1.08	1.05	91.2
D. crenata var. *crenata* f. *pubescens* (78)	3-colporate	prolate spheroidal	reticulate	semitectate	21.0	16.9	19.0	0.92	19.4	16.8	18.4	0.69	1.03	99.6
D. crenata var. *floribunda* (78)	3-colporate	prolate spheroidal	reticulate or microreticulate	semitectate	20.1	17.0	18.3	0.80	18.2	14.5	16.9	0.87	1.08	99.4
D. gracilis var. *gracilis* (26)	3-colporate	prolate spheroidal	microreticulate	semitectate	19.2	16.0	17.5	0.89	18.5	15.5	16.7	0.72	1.05	92.0
D. gracilis var. *microcarpa* (26)	3-colporate	prolate spheroidal	microreticulate	semitectate	17.9	15.5	16.7	0.57	17.1	14.9	15.9	0.56	1.05	98.2
D. gracilis var. *ogatae* (?)	3-colporate	prolate spheroidal	reticulate or microreticulate	semitectate	21.5	17.5	19.4	0.88	20.2	16.2	18.3	1.09	1.06	98.6
D. gracilis var. *zentaroana* (52)	3-colporate	prolate spheroidal	microreticulate	semitectate	19.7	17.3	18.2	0.59	19.5	17.1	17.9	0.66	1.02	98.6
D. maximowicziana (26)	3-colporate	prolate spheroidal	reticulate	semitectate	18.0	15.4	16.6	0.59	17.9	15.2	16.4	0.60	1.02	96.8
D. naseana var. *naseana* (52)	3-colporate	prolate spheroidal	reticulate	semitectate	19.0	16.0	17.5	0.75	18.8	15.2	17.2	0.83	1.05	98.8
D. scabra var. *scabra* (26)	3-colporate	prolate spheroidal	microreticulate	semitectate	16.8	14.8	15.5	0.56	16.1	14.5	15.2	0.45	1.02	96.2
D. scabra var. *sieboldiana* (26)	3-colporate	prolate spheroidal	microreticulate	semitectate	17.0	14.8	15.6	0.58	16.0	14.5	15.2	0.41	1.02	95.8
D. uniflora (26)	3-colporate	subprolate	reticulate or microreticulate	semitectate	23.1	19.9	21.5	0.75	18.9	16.1	17.5	0.64	1.23	98.6
D. yaeyamensis (26)	3-colporate	prolate spheroidal	reticulate or microreticulate	semitectate	18.1	16.0	17.3	0.46	17.1	14.2	15.4	0.60	1.13	91.4


24
22
20
18
16
14
15 16 17 18 19 20 21 22 23 24 25
equatorial axis μm
polar axis μm
● D. crenata var. crenata f. crenata (2n=130)
▲ D. crenata var. floribunda (2n=78)
▼ D. crenata var. crenata f. pubescens (2n=78)


20
18
16
14
15 17 19 21 23
equatorial axis μm
polar axis μm
● D. gracilis var. ogatae (?)
▲ D. gracilis var. zentaroana (2n=52)
▼ D. gracilis var. gracilis (2n=26)
■ D. gracilis var. microcarpa (2n=26)

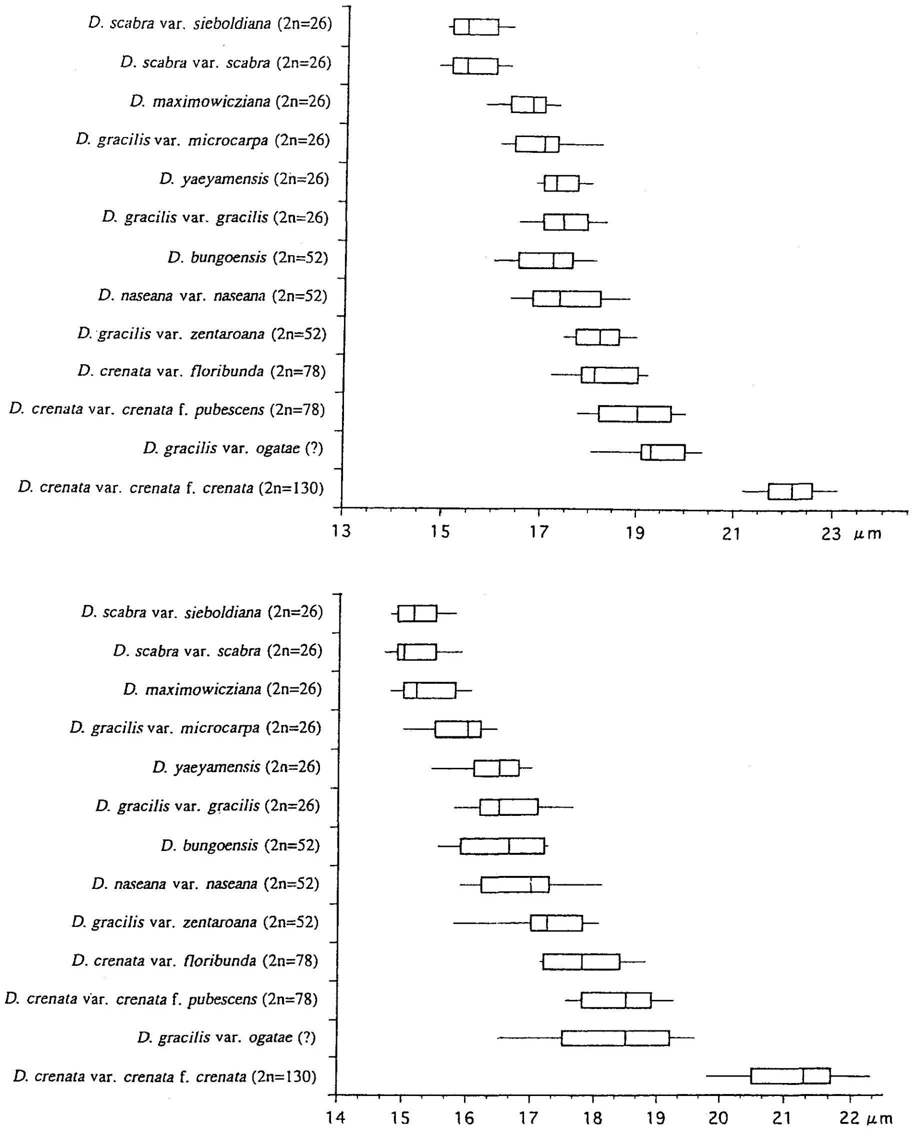


Fig. 58. Box plot of the size of polar axis (upper) and equatorial axis (lower) of fully stained pollen grains collected from diploids, tetraploids, hexaploids, decaploids of Japanese *Deutzia*. The box part emcompasses the 25 th–75 th percentile points of the data. The vertical lines with in the boxes represent medians.

Fig. 57. Scatter diagram showing mean of the size of polar axis and equatorial axis of pollen in the hexaploid and decaploid of *Deutzia crenata* (upper) and scatter diagram showing mean of the size of polar axis and equatorial axis of pollen in the diploid and tetraploid of *Deutzia gracilis* (lower).

and equatorial axis; 16.9 μm to 18.4 μm and the decaploid, *D. crenata* var. *crenata* f. *crenata*, polar axis; 22.2 μm and equatorial axis; 21.1 μm.

Discussions

Ohba (1989) classified Japanese *Deutzia* into seven species with seven varieties and six forms and two hybrids. Three species, *D. crenata*, *D. gracilis* and *D. scabra*, which are widely distributed throughout Japan westwards from Honshu, are extremely variable in morphology such as the shape and size of leaves, trichome, inflorescences, flowers, and also the habit. In this study, the morphology and fertility of pollen grains, and also the correlation with ploidy level were observed. Our results indicated that the pollen grains of all the taxa are 3-colporate, and except *D. uniflora* they are prolate spheroidal, while those of *D. uniflora* are subprolate. Their exine is semitectate and the columellae are unbranched or indistinctly branched. The tectum-pattern is reticulate or microreticulate. A comparison suggests that there are no essential differences in the exile structure within or between the species.

However, the pollen grains show a wide range of variation in the size. It is found that the pollen size correlates with ploidy level. The correlation is clear among the species examined and also among intraspecific taxa within a single species showing variations in ploidy levels.

Such a correlation between ploidy level and pollen size has been pointed out by some works in herbaceous plants (Darlington 1932, Grant 1971). The present study reveals that the same tendency may occur in woody plants. The size of pollen grains of *D. gracilis* var. *ogatae* is the largest in *D. gracilis*. Though the chromosome number of var. *ogatae* has not been reported, it is presumed that the variety is hyperploid.

All taxa examined, including *D. bungoensis* which is inferred as a natural hybrid between *D. crenata* var. *floribunda* and *D. scabra* var. *sieboldiana* (Hatusima 1954), produced normal uniform pollen grains with high stainability over 90 %. The direct contact of pollen sterility in polyploidy taxa has not been find.

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鈕　力明，大場秀章：**日本産ウツギ属（ユキノシタ科）の系統分類学的研究　2．花粉粒**

日本産ウツギ属（ユキノシタ科）の8種とその5変種1品種について，花粉粒の形態，大きさ，染色率及び倍数性と花粉の大きさの相関について観察を行った．花粉の形態は3溝型で，長球状球型又は亜長球型であった．表面模様は網目紋又は微網状紋で，外壁は，上部で枝分かれするかまたは未分枝の柱からなる柱状層をもち，外表層はやや発達する半外表層型であった．染色体の倍数化と花粉の大きさの相関を検討した結果，ウツギ（*D. crenata*）とヒメウツギ（*D. gracilis*）の種内分類群では，倍数性が高いほど花粉のサイズが大きい傾向があることが分った．このような傾向は種間にも存在することが分った．このことからアオコウツギ（*D. gracilis* var. *ogatae*）の染色体数は，まだ未報告であるが花粉の大きさから高次倍数体であると推定される．観察した各分類群の花粉の染色性はどの倍数体においても90％以上と高く，高次倍数化に伴う稔性の低下はないと考えられる．

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